PB84-206887

Mutagenic Agents

(U.S.) Environmental Protection Agency
Washington, DC

Jun 84

U.S. Department of Commerce
National Technical Information Service
NTIS

PB84-206887

EPA-600/D-84-152
June 1984

MUTAGENIC AGENTS

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WASHINGTON, DC 20460

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SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/D-84-152	2.
3. RECIPIENT'S ACCESSION NO. PB84 206887	
4. TITLE AND SUBTITLE Mutagenic Agents	5. REPORT DATE June 1984 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Sidney Greene, Ph.D. Food and Drug Administration K.S. Lavappa, Ph.D., Environmental Protection Agency	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Reproductive Effects Assessment Group (RD-689) Office of Health and Environmental Assessment U.S. Environmental Protection Agency 401 M St., SW, Washington, DC 20460	10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO. N/A
12. SPONSORING AGENCY NAME AND ADDRESS Same as (9) above	13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA/600/21
15. SUPPLEMENTARY NOTES Project Officer: K.S. Lavappa	

16. ABSTRACT

A description of some chemicals that are used in chemotherapy and pyschotherapy is presented in relation to their mutagenic activity. A comparison of the mutagenic activity of these pharmaceutical compounds together with some industrial chemicals is also made to understand their relationship with teratogenicity and carcinogenicity. The majority of these chemicals exhibited a good correlation between mutagenicity, carcinogenicity, and teratogenicity.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT NTIS - Distribute to the public	19. SECURITY CLASS *(This Report)* Unclassified 20. SECURITY CLASS *(This page)* Unclassified	21. NO. OF PAGES 22 22. PRICE

EPA Form 2220-1 (9-73)

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MUTAGENIC AGENTS

DEFINITION OF MUTATION

Mutations are heritable alterations in the genetic material, deoxyribonucleic acid (DNA), of an organism and may occur in both somatic and germinal cells. Somatic mutations may lead to various diseases in the organism, whereas germinal mutations are transmitted from generation to generation. Mutations can be dominant or recessive in nature. They can occur spontaneously or can be induced by chemicals, radiation, and viruses. There are two types: point mutations and chromosomal aberrations. The mutagenic effects of some antineoplastic agents, phenothiazine tranquilizers, industrial chemicals, as well as several other agents are presented as examples. The carcinogenic and teratogenic effects of some of these are briefly discussed. Inasmuch as the carcinogenicity and teratogenicity may originate via mutation, a discussion of the evidence supporting this view is also provided.

TYPES OF MUTATIONS

Point Mutations

Point mutations or gene mutations involve one or a few base-pairs of the DNA of an organism. They can be classified as base-pair substitution and frameshift mutation, also known as addition and deletion.

Base-Pair Substitution--

With respect to base-pair substitution, two types occur: transitions and transversions. If the substitution involves purine for purine or pyrimidine for pyrimidine, then the mutation is called a transition. On the other hand, if the substitution involves purine for pyrimidine or pyrimidine for purine, then the mutation is called a transversion.

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Frameshift or Addition and Deletion--

Frameshift mutations involve addition or deletion of base-pairs on the DNA. These involve large and small macrolesions. Frameshift mutations are readily reverted by many mutagens.

Chromosomal Aberrations

Chromosomal aberrations can be classified into structural aberrations and numerical aberrations. Structural aberrations result from breakage of chromosomes and reunion of broken ends. Numerical aberrations are changes in the number of chromosomes resulting from errors in the movement of chromosomes during mitosis or meiosis mainly as a consequence of spindle malfunctions.

Structural Aberrations--

There are two major types of structural aberrations, namely, chromosome-type and chromatid-type. The chromosome-type is produced when mutagens act in the G_1 phase of the cell cycle where the sites of breakage and reunion involve the entire chromosome. The chromatid-type is produced when mutagens exert their effect in the S or G_2 phase of the cell cycle in which each chromosome has already divided into two chromatids. Some alkylating chemicals produce chromosomal aberrations regardless of the stage of the mitotic cycle at which the cell is exposed.

Structural aberrations include the following:

1. Chromosome break: a discontinuity in both arms of the chromosome.
2. Chromatid break: a discontinuity in one arm of the chromosome.
3. Deletion: paired acentric fragments resulting from a single chromosome break.
4. Minutes: small acentric fragments of chromosomes or chromatids.

5. Acentric rings: chromosome with a ring-like configuration lacking a centromere.

6. Centric rings: chromosome with a ring-like configuration and a centromere.

7. Asymmetrical interchange: exchange of material between chromosomes resulting in dicentric chromosomes (chromosomes with two centromeres).

8. Symmetrical interchange: exchange of material between chromosomes resulting in a quadriradial configuration.

Numerical Aberrations--

This category includes trisomics, monosomics, and polyploidy. Trisomics and monosomics result when both chromosomes of a pair undergo nondisjunction during cell division. If nondisjunction occurs during meiosis, then the resulting two gametes will be aneuploids, i.e., one gamete will gain one extra chromosome and the other gamete will lose one chromosome. When the gamete containing an extra chromosome is fertilized by a normal gamete from the opposite sex, the resulting zygote will be trisomic. On the other hand, when the gamete lacking a chromosome is fertilized by a normal gamete from the opposite sex, the resulting zygote will be monosomic. Nondisjunction of chromosomes during mitosis (somatic cells) may lead to diseases such as cancer. Continued duplication of chromosomes within the nucleus without accompanying cell division leads to polyploidy.

EFFECTS THAT MAY ORIGINATE VIA MUTATION

Carcinogenicity

The process of carcinogenesis may involve mutations as primary events or as multimedia events. There is a great deal of evidence indicating that carcinogens, such as chemicals, radiation, and viruses, are also mutagens causing genetic changes (60). Studies of chemical carcinogenesis (4), radiation carcinogenesis (41), and dominantly inherited tumors (37) have suggested that mutations are involved in cancer at possibly two stages.

Altered patterns of chromosomes, both structural and numerical, and mitotic anomalies have been noted in many malignant cells. However, there are some tumors that do not show altered patterns of chromosomes. Consequently, it is not clear whether chromosome alteration in tumor cells is a cause or a consequence of cancer.

Teratogenicity

Some chemical agents are teratogenic in nature and induce congenital abnormalities in experimental animals. Many of these teratogens are also mutagenic in nature and produce gene mutations and chromosomal aberrations. Chemical agents that produce both teratogenic and mutagenic effects include cyclophosphamide (CP), methylmethanesulfonate (MMS), and ethylmethanesulfonate (EMS). When administered to pregnant mice, 6-aminoicotinamide, a vitamin antagonist, induces both cleft palate and chromosomal aberrations in the cleft palate tissue of the fetus (38). However, some teratogenic agents, such as chlorambucil and griseofulvin, do not induce mutations (31). The relationship between teratogenicity and mutagenicity is still not clearly understood.

In the human population, some congenital abnormalities are inherited as Mendelian genes (40). Some other types of congenital abnormalities, such as Down's Syndrome, Cri-du-Chat Syndrome, Turner's Syndrome, and Kleinfelter's Syndrome are associated with specific chromosomal alterations (63).

MUTAGENIC AGENTS

Mutagenic agents are defined as those physical or chemical agents capable of significantly increasing mutational events in experimental animals, thereby posing a possible danger to humans. Many chemical mutagens are also carcinogens and teratogens (Table 1).

Antineoplastic Agents

Antineoplastic agents are used clinically in the treatment of many forms of cancer. The rationale for their use is that they exert their effects on various cellular processes, particularly those controlling the division of cancerous cells. Antineoplastic agents have also been found to be mutagenic, carcinogenic, and teratogenic in experimental animals.

Triethylenemelamine--

Triethylenemelamine (TEM) is an alkylating agent which produces its effects by cross-linking with DNA. It is mutagenic in a variety of systems. Chromosomal aberrations have been demonstrated in cultured human lymphocytes treated with TEM. Translocations and chromosome fragmentation in spermatocytes of mice treated with TEM have also been observed. TEM induces dominant lethal mutations in rats, mice, and heritable translocations in the F_1 progeny of treated male mice. The mutagenic activity of TEM has been reviewed by Fishbein, Flamm, and Falk (16). Intraperitoneal and subcutaneous administration of TEM in mice results in the development of pulmonary tumors. Similarly, rats treated with TEM develop a high incidence of sarcomas (62).

TEM is also a potent teratogen, and is embryolethal in the rat. Intraperitoneal injection of TEM at 0.3 to 0.6 mg/kg in the rat on the 11th to 12th day of pregnancy produces defects in the central nervous system, palate, and skeleton. Growth retardation, enlargement of the myocele, and somite degeneration have also been reported in mouse embryos (30).

Cyclophosphamide--

Cyclophosphamide is an antitumor agent widely used in cancer chemotherapy (39). It is also effective in the treatment of rheumatoid arthritis and Wegner's granulomatosis.

Cyclophosphamide has been shown to be mutagenic in a variety of systems, requiring metabolic activation before producing mutagenic by-products. Chromatid aberrations in treated laboratory mammals and in human lymphocyte cultures following cytoxan therapy have been detected (17). Dominant lethal mutations and heritable translocations are also induced by cyclophosphamide. Induction of micronuclei in the bone marrow cells of laboratory rodents and the induction of univalents during meiosis have also been attributed to the action of cyclophosphamide. Intraperitoneal administration of cyclophosphamide to mice and rats produces pulmonary tumors, reticular cell sarcomas, and lymphomas. Weisburger has recently reviewed the carcinogenic properties of some drugs used in clinical cancer chemotherapy, including cyclophosphamide (66).

Cyclophosphamide is a potent teratogen, inducing skeletal defects, cleft palate, and exencephaly in rats treated with 7 to 10 mg/kg of the drug on the 11th and 12th days of pregnancy (7). It has also been shown to be teratogenic in mice and humans.

6-Mercaptopurine--

6-Mercaptopurine induces dominant lethal mutations in mice. Increases in the frequency of chromatid and isochromatid deletions 14 and 15 days after treatment with 150 or 250 mg/kg of 6-mecaptopurine in cells analyzed at the diakinesis-metaphase 1 stage of spermatogenesis of mice have also been noted (19). A high incidence of lymphomas was found in mice treated intraperitoneally with 6-mercaptopurine (13).

This SH-containing purine analogue, and its derivatives, are teratogenic in the chick producing facial defects.

Vincristine--

Vincristine, an alkaloid derived from the plant Vinca rosea, is a spindle poison producing a colchicine-like effect. It is used in cancer chemotherapy. It induces micronuclei and polyploidy in bone marrow cells (51). At lower doses the spindle disruption is partial, but at higher doses the disruption is drastic and leads to polyploid cells. Patients treated with vincristine for Hodgkin's disease develop malignancies such as acute leukemia (1).

This antineoplastic agent is teratogenic in hamsters, rats, and monkeys. Intravenous injection of 0.1 mg/kg of vincristine to Syrian hamsters on the 8th gestational day produces skeletal and eye defects (14). Similar effects have been found in rats. Teratogenic effects of vincristine have also been demonstrated in the rheus monkey.

Mitomycin-C--

Mitomycin-C, an antineoplastic antibiotic used in cancer chemotherapy, has been found to be mutagenic. Human lymphocyte cultures treated with Mitomycin-C exhibited chromatid aberrations and chromosome rearrangements (18).

Subcutaneous administration of mitomycin-C to mice (23) and intraperitoneal administration of mitomycin-C to rats (67) induced a high incidence of sarcomas. This growth inhibitor isolated from Streptomyces caespitosus produces skeletal defects of the palate and brain of the mouse fetus (59).

Actinomycin-D--

Actinomycin-D, a chromopeptide antibiotic, has been uses as an antieoplastic agent. Biochemically, it is known to suppress DNA-dependent

RNA synthesis and binds to DNA as revealed by tritium-labeled autoradiography. Actinomycin-D also induces inhibition of mitosis and chromosome breakage in Chinese hamster cells in vitro (49). Chromosome damage (such as chromatid breakages and rearrangements) has also been detected in human lymphocytes, skin, and HeLa cultures (44). Chromatid aberrations appear to be preferentially present in the centromeric areas.

Induction of sarcomas in two strains of mice by subcutaneous injection of actinomycin-D has been demonstrated (33). The drug has also been shown to induce mammary carcinomas, hepatomas, and squamous cell carcinomas in DBA and Swiss albino mice when administered either subcutaneously or intraperitoneally (12). Similarly, fibrosarcomas in Fischer rats were induced by the intraperitoneal or intravenous injection of actinomycin-D.

Actinomycin-D is teratogenic in rats when administered daily for the first 10 days of pregnancy. Teratogenic effects include defects of the nervous system and bronchial arch malformations (61). Similar effects have been noted in rabbits.

Phenothiazine Tranquilizers

Phenothiazine tranquilizers are widely used in the treatment of psychiatric diseases. Studies in animals and humans have indicated that these tranquilizing drugs are also mutagenic in nature.

Chlorpromazine--

Chlorpromazine forms stable free radicals when photoactivated by ultraviolet light; these free radicals intercalate with DNA (5). Studies on the frequencies of chromosome aberrations in patients treated with chlorpromazine showed variations beginning from no change (8) to increases

in aberrations (43). Recently, Kelly-Gavert and Legator (34) have clearly demonstrated that chromosome aberrations and point mutations can be induced in Chinese hamster cells following treatment of cultures with UV-irradiated chlorpromazine. Induction of sister chromatid exchanges (SCEs) following treatment of Chinese hamster cells with chlorpromazine and visible light has also been demonstrated. The general consensus is that chlorpromazine is nonteratogenic (32).

Triflupromazine--

Induction of chromosome breakage and rearrangements in cultured rat kangaroo cells treated with triflupromazine has been demonstrated. Inhibition of mitosis was also noted in rat kangaroo cells treated with 20 ug/ml of the drug for 3 days (20). Dose-dependent increases in dominant lethal mutations are also induced by triflupromazine (47).

SUMMARY

Mutations are changes in the sequence of the genetic material of prokaryotic and eukaryotic organisms. Most mutations are deleterious to the organism producing both quantitative and qualitative changes. Heritable gonadal mutations are far more serious than somatic mutations. A variety of environmental insults have been known to cause both point mutations and chromosomal aberrations. Mutagens produce their effects by a variety of means all of which adversely affect DNA; thus, they may also be carcinogenic and teratogenic.

Some chemotherapeutic and psychotherapeutic agents may be capable of producing these effects. Antineoplastic agents (triethylenemelamine, cyclophosphamide, 6-mercaptopurine, vincristine, actinomycin-D, and

mitomycin-C) induce mutagenic, carcinogenic, and teratogenic effects in experimental animals. Phenothiazine tranquilizers (chlorpromazine and trifluprom azine) produce chromosomal aberrations in cultured cells and dominant lethal mutations in mice.

These agents are not the only chemicals capable of producing mutagenicity. Table 1 shows that a variety of chemicals are able to produce mutagenicity, carcinogenicity, and teratogenicity. The correlation among an agent's ability to produce mutagenicity, carcinogenicity, and teratogenicity appears extremely good for the antineoplastic agents. This should not be surprising for these agents have a clearly defined site of action in DNA. Very little definitive information with respect to this correlation can be gleaned from the small number of chemicals selected in this table. A general statement, however, may be in order: agents that have as their site of action, DNA, are usually mutagens, teratogens, or carcinogens. It certainly is evident that a chemical may share more than one of these properties. The mechanism of action as listed in Table 1 refers to the reported means by which the agent causes mutagenic damage.

TABLE 1. COMPARISON OF CHEMICALS FOR CARCINOGENICITY, MUTAGENICITY, AND TERATOGENICITY

Chemical	Mechanism of Action	Mutagenicity*	Teratogenicity*	Carcinogenicity*
Antineoplastic Agents:				
triethylenemelamine	alkylating agent	+(16)	+(30)	+(62)
cyclohosphamide	alkylating agent	+(17)	+(7)	+(66)
6-mercaptopurine	base analog	+(19)	+(6)	+(13)
vincristine	c-mitotic agent	+(51)	+(14)	+(1)
mitomycin-C	alkylating agent	+(18)	+(59)	+(23,67)
antinomycin-D	intercalating agent	+(44)	+(61)	+(12,33)
Phenothiazines:				
chlorpromazine	arylating agent	+(34)	-(32)	0
trifluprom azine	arylating agent	+(20,47)	0	0
Antiepileptics:				
phenytoin	not certain	+(50)	+(15)	+(28)
Schistosomacides:				
hycathone	intercalating agent	+(21)	+(42)	+(27)
Naturally Occurring Substances:				
caffeine	base analog	+(36)	+(9)	+(58)

*Numbers in parentheses denote reference: (+) denotes significant activity; (-) deontes nonsignificant activity; (±) ambigous results; (0) information lacking; and (?) questionable results.

(continued on the next page)

TABLE 1. (continued)

Chemical	Mechanism of Action	Mutagenicity*	Teratogenicity*	Carcinogenicity*
Industrial Chemicals:				
benzene	not certain	+(48)	+(64)	±(26)
DDT, pp'	not certain	+(46)	-(45)	+(25)
formaldehyde	alkylating agent	+(55)	-(52)	+(57)
TCDD-2,3,7,8-tetrachloro-dibenzo-p-dioxin	intercalating agent	±(22)	+(10)	?(29)
methyl mercury	not certain	+(54)	+(35)	0
cadmium compounds	not certain	+(11,53,65)	+(3)	+(24)
ethyl alcohol	not certain	+(2)	+(56)	0

*Numbers in parentheses denote reference: (+) denotes significant activity; (-) denotes nonsignificant activity; (±) ambiguous results; (0) information lacking; and (?) questionable results.

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